

Claims

1. A method for producing fibrinogen comprising:
providing a first DNA segment encoding a secretion signal operably linked to a fibrinogen $\text{A}\alpha$ chain, a second DNA segment encoding a secretion signal operably linked to a fibrinogen $\text{B}\beta$ chain, and a third DNA segment encoding a secretion signal operably linked to a fibrinogen γ chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in the mammary gland of a host female mammal;

introducing said DNA segments into a fertilized egg of a non-human mammalian species;

inserting said egg into an oviduct or uterus of a female of said species to obtain offspring carrying said DNA constructs;

breeding said offspring to produce female progeny that express said first, second and third DNA segments and produce milk containing biocompetent fibrinogen encoded by said segments;

collecting milk from said female progeny;
and recovering the fibrinogen from the milk.

2. A method according to claim 1 wherein said species is selected from the group consisting of sheep, pigs, goats and cattle.

3. A method according to claim 1 wherein each of said first, second and third DNA segments comprises an intron.

4. A method according to claim 1 wherein the molar ratio of said first, second and third DNA segments is within the range of 0.5-1:0.5-1:0.5-1.

5. A method according to claim 1 wherein each of said first, second and third DNA segments is operably linked

to a transcription promoter selected from the group consisting of casein, β -lactoglobulin, α -lactalbumin and whey acidic protein gene promoters.

6. A method according to claim 1 wherein said first, second and third DNA segments are expressed under the control of a β -lactoglobulin promoter.

7. A method according to claim 1 wherein said introducing step comprises injecting said first, second and third DNA segments into a pronucleus of said fertilized egg.

8. A method according to claim 1 wherein said fibrinogen is human fibrinogen.

9. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 470 to nucleotide 8100.

10. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 512 to nucleotide 8100.

11. A method of producing fibrinogen comprising:
incorporating a first DNA segment encoding a secretion signal operably linked to an $A\alpha$ chain of fibrinogen into a β -lactoglobulin gene to produce a first gene fusion;
incorporating a second DNA segment encoding a secretion signal operably linked to a $B\beta$ chain of fibrinogen into a β -lactoglobulin gene to produce a second gene fusion;
incorporating a third DNA segment encoding a secretion signal operably linked to a γ chain of fibrinogen into a β -lactoglobulin gene to produce a third gene fusion;
introducing said first, second and third gene fusions into the germ line of a non-human mammal so that said DNA segments are expressed in a mammary gland of said mammal

or its female progeny and biocompetent fibrinogen is secreted into milk of said mammal or its female progeny ;
 obtaining milk from said mammal or its female progeny; and
 recovering said fibrinogen from said milk.

~~13~~ 12. A method according to claim ~~11~~ ^{Cow} wherein said mammal is a sheep, pig, goat or ^{bovine}.

~~14~~ 13. A method according to claim ~~11~~ wherein each of said first, second and third gene fusions comprises an intron.

~~15~~ 14. A method according to claim ~~11~~ wherein the molar ratio of said first, second and third gene fusions introduced is within the range of 0.5-1:0.5-1:0.5-1.

~~16~~ 15. A method according to claim ~~11~~ wherein said introducing step comprises injecting said first, second and third gene fusions into a pronucleus of a fertilized egg and inserting said egg into an oviduct of a pseudopregnant female to produce female offspring carrying said gene fusions in the germ line.

~~17~~ 16. A method for producing fibrinogen comprising:
 providing a transgenic female non-human mammal carrying in its germline heterologous DNA segments encoding $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen, wherein said segments are expressed in a mammary gland of said mammal and fibrinogen encoded by said segments is secreted into milk of said mammal;

collecting milk from said mammal; and
 recovering said fibrinogen from said milk.

~~18~~ 17. A method according to claim ~~16~~ ^{Cow} wherein said mammal is a sheep, pig, goat or ^{bovine}.

A ~~18.~~ nucleus A non-human mammalian embryo containing in its heterologous DNA segments encoding $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen.

B ~~19.~~ A transgenic non-human female mammal that produces recoverable amounts of human fibrinogen in its milk.

B ~~20.~~ A process for producing a transgenic offspring of a mammal comprising:

providing a first DNA segment encoding a fibrinogen $\text{A}\alpha$ chain, a second DNA segment encoding a fibrinogen $\text{B}\beta$ chain, and a third DNA segment encoding a fibrinogen γ chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in a mammary gland of a host female mammal and secretion into milk of said host female mammal;

introducing said DNA segments into a fertilized egg of a mammal of a non-human species;

inserting said egg into an oviduct or uterus of a female of said non-human species to obtain an offspring carrying said first, second and third DNA segments.

C ~~21.~~ A process according to claim ~~20~~ ²³ wherein said offspring is female.

C ~~22.~~ A process according to claim ~~20~~ ²³ wherein said offspring is male.

C ~~23.~~ A non-human mammal produced according to the process of claim ~~20~~ ²³.

C ~~24.~~ A non-human mammal according to claim ~~23~~ ²⁶ wherein said mammal is female.

C ~~25.~~ ²⁶ non-human female mammal according to claim ~~24~~ ²³ that produces milk containing biocompetent fibrinogen encoded by said DNA segments.

27
26. A non-human mammal according to claim 23
wherein said mammal is male.

27
28 A non-human mammal carrying in its germline
DNA segments encoding ^{human} heterologous α , β and γ chains of
fibrinogen, wherein female progeny of said mammal express
said DNA segments in a mammary gland to produce biocompetent
fibrinogen.

29 ³¹ _{non-human}
A mammal according to claim 27 wherein said
mammal is female.

30
29 ³² _{non-human}
A mammal according to claim 27 wherein said
mammal is male.

Add B³

94